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Individual-level air pollutants exposure and pregnancy outcomes in patients who underwent assisted reproductive technology: A retrospective longitudinal cohort study

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Abstract

Background: The effects of air pollution on the results of assisted reproductive technology (ART) treatment in terms of pregnancy outcomes were inconclusive.

Methods: We performed an individual-level retrospective longitudinal cohort study among 11,968 participants undergoing ART treatment from a general hospital in Hefei, China in 2013-20. Monthly mean concentrations of air pollutants [fine particulate matter ($PM_{2.5}$), suspended particulate matter (PM_{10}), ozone (O_3), sulfur dioxide (SO_2), Nitrogen dioxide (NO_2) and carbon oxide (CO)] during 3 Periods were obtained from ChinaHighAirPollutants (CHAP) dataset: respective Periods 1, 2 and 3 refer to 90 days, one or two years prior to oocyte retrieval. Multiple logistic regression model was applied to explore the impact of air pollution on four ART results (biochemical pregnancy, clinical pregnancy, pregnancy loss, and live birth).

Results: We observed negative relationships of PM_{10}, $PM_{2.5}$, CO and SO_2 exposures with pregnancy outcomes were more evident during one year exposure (Period 2). An interquartile range increment of ambient PM_{10}, $PM_{2.5}$, CO, and SO_2 exposures during Period 2 was associated with respective decrements of 5.85% (aOR: 0.94, 95% CI: 0.90–0.99), 7.82% (aOR: 0.92, 95% CI: 0.88–0.97), 10.60% (aOR: 0.89, 95% CI: 0.82–0.93) and 12.38% (aOR: 0.88, 95% CI: 0.82–0.93) on clinical pregnancy, while O_3 and NO_2 showed positive associations. Associations were stronger in patients undergoing frozen embryo transfer, aged < 32 years, with normal BMI, employed status, one embryo transferred, and in warm season.

Conclusions: Our data show that long-term individual-level exposure to the air pollutants of PM$_{10}$, PM$_{2.5}$, CO, SO$_2$ but not O$_3$ or NO$_2$, especially in one year exposure before oocyte retrieval, could have a negative impact on ART outcomes.

Keywords: air pollution, assisted reproduction technology, retrospective study, pregnancy outcome, logistic regression

1. Introduction

Air pollution has been a long-standing problem and remains a major public health challenge, which has long been recognized as a significant harmful environmental stimulus for cardiopulmonary diseases.[1-3] In practice, it is only during recent years, the reproductive health impacts perturbed by air pollution become a hot research topic in environmental epidemiology.[4,5] The adversely reproductive health effects induced by air pollution can result in lower human fertility,[6-9] pregnancy loss,[10] preterm birth,[11] fetal growth restriction[12] and stillbirth.[13] Existing evidence regarding relationships between air pollutants exposure and infertility among couples who are attempting to conceive naturally has indicated that pre-gestational or gestational exposure to fine particulate matter ($PM_{2.5}$) is associated with decreased fertility and increased pregnancy loss.[14-16]

In recent years, the infertility rate is climbing gradually. According to a new report published by World Health Organization (WHO) in 2023, about 17.5% of the adult population (roughly 1 in 6 globally) experience infertility.[17] In China, according to the National Bureau of Statistics, the rate of infertility in 2021 is estimated to be as high as 12%-18%, and one epidemiological investigation performed in eight provinces in China reported that the infertility rate is up to 25% among couples of reproductive age.[18] This fact leads a rising number of infertility couples who wish to become parents to turn to the assisted reproductive technology (ART) treatment. Evidence of ART for infertility treatment is currently sufficient,[19] and its main approaches include in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI). In the past, researchers merely focus on the impacts of clinical relative issues on infertility and ART while they pay little attention to the environmental stimulus such as ambient air pollutants.

Despite that some publications have linked air pollution with high risks of adverse pregnancy outcomes,[20,21] evidence on such impacts in ART patients is limited and mixed.[22,23] Some reported adverse effects on IVF outcomes,[24-27] whereas others observed non-significant or opposing results.[28-31] For example, Wu and colleagues detected that air pollution [O_3, nitrogen dioxide (NO_2), sulfur dioxide (SO_2), and carbon monoxide (CO)] was adversely associated with pregnancy and live birth in patients receiving IVF treatment.[25] Li and colleagues reported associations between particles with diameter ≤2.5 μm ($PM_{2.5}$), particles with diameter ≤10 μm (PM_{10}), NO2, SO2, and CO exposures and decreased probability of clinical pregnancy.[27] In contrast, Gaskins et al. reported null associations of short-term and long-term exposures to NO_2, O_3, and $PM_{2.5}$ with pregnancy loss in patients with IVF treatment.[30] Quraishi et al. detected exposure to PM_{10}, $PM_{2.5}$, and NO_2 was non-significant associated with pregnancy loss and livebirth.[31] The inconsistent results are also found in investigations assessing ambient O_3 exposure.[28] Collectively, the possibly heterogeneous effects of air pollution on ART pregnancy outcomes remain to be elucidated.

Up to now, the majority of previous research has considered a relatively short exposure window, which is liable to neglect the chronic exposure period. Liu and colleagues designed seven different time windows to explore the correlation of air pollution and IVF outcomes, while the longest window only explored up to 85 days before oocyte retrieval.[23] Less research, meanwhile, has considered the role of frozen embryo transfer (FET) cycles in associations of air pollution with pregnancy outcomes. Presently, if it occurs a failed fresh transfer or freeze-all cycle, according to the acknowledged standard clinical practice, FET cycles treatment will be performed again after one menstrual cycle at least.[32] Besides, compare to spontaneous conception and FET, patients receiving fresh transfer cycles are more easily to be experienced damaged endometrial receptivity and embryo implantation, and high odds of preterm birth and lower birthweight.[33]

Therefore, we performed this individual-level retrospective longitudinal cohort study in Hefei, Anhui Province, China to comprehensively investigate the effects of monthly exposure to six criteria air pollutants on pregnancy outcomes among women undergoing IVF or ICSI cycle and meanwhile explore the possible effect modifiers.

2. Methods

2.1 Study Design

This retrospective longitudinal cohort study was performed in 15,414 participants who underwent assisted reproductive technology (ART) between 1 February 2013 and 31 December 2020 in the Center for Reproductive Medicine of 901st Hospital of PLA Joint Logistic Support Force, a large general hospital in Hefei, Anhui Province, China. Some patients were excluded from the final analysis following the criteria below: (i) patients over 45 years old, (ii) twin or higher-order multifetal pregnancies, (iii) patients undergoing artificial insemination, (iv) patients without undergoing embryo transfer after oocyte retrieval; or (v) patients with no medical examination or precise residential address. Finally, we included a total of 11,968 singleton patients for biochemical pregnancy and clinical pregnancy outcomes in statistical analysis. Among 4,673 clinical pregnancy patients, 19 patients who were loss of follow-up were excluded, and thus 4,654 patients were finally included for the remaining two (pregnancy loss and live birth) in statistical analysis. Details of subjects included in the current study are presented in Figure 1.

Data on female age, body mass index (BMI), educational level, employment status, residential address, duration and type of infertility, controlled ovarian stimulation protocols, number of retrieved oocytes, fertilization method, date of embryo transfer, and type/number/quality of transferred embryos were extracted from the medical records. Gardner blastocyst scoring was used to conduct blastocysts quality assessment and the details were shown in Supplemental Methods[34].

2.2 IVF or ICSI procedure

All subjects received IVF or ICSI treatment with following a standard protocol at the center. Generally, the overall IVF process included four steps among which controlled ovarian stimulation (COS) was the first step, followed by oocyte retrieval, embryo transfer, and pregnancy tests. For COS, the following protocols including the long gonadotrophin-releasing hormone (GnRH)- agonist (-a) protocol, short GnRH-agonist protocol, GnRH antagonist (-ant) protocol, hormone replacement treatment protocol, and others (mild stimulation protocol, natural protocol and so on) were decided by the physician based on the patient's individual characteristics (i.e., age, ovarian function and medical history of IVF). Ovulation was induced by injecting human chorionic gonadotropin (hCG) if the physician observed three or more follicles ⩾ 18 mm in diameter, and the oocytes were then retrieved 34–36 h later.

The main methods of ART include IVF and ICSI. A single sperm was injected into the egg under a microscope by using the ICSI procedure. IVF or intracytoplasmic sperm injection (ICSI) was performed following the clinical indication and sperm quality. In the fresh cycles, one or two or three embryos were transferred three or five days after oocyte retrieval. The remaining good-quality embryos were cryopreserved. In frozen cycles, patients received a natural protocol or a programmed cycle regimen for endometrial preparation and then the frozen embryos were thawed and the same transfer procedure employed for fresh embryo transfer was used to transfer. Additionally， progesterone was used for luteal support after oocyte retrieval.[21,25,35]

2.3 Health Outcomes

In our study, we included four health outcomes, including biochemical pregnancy, clinical pregnancy, live birth, and gestation pregnancy loss. Among them, we defined (i) biochemical pregnancy as blood levels of human chorionic gonadotropin (HCG) ⩾ 20 mIU/ml after 14 days of embryo transfer, which can determine early pregnancy, does not mean biochemical abortion; (ii) clinical pregnancy as the identification of an ultrasound-confirmed gestational sac after 30 days of embryo transfer; (iii) live birth as the delivery of at least one live-born infant after 28 weeks of gestation; and (iv) gestation pregnancy loss as the termination of pregnancy prior to 28 weeks.

2.4 Exposure Data

Monthly air pollution data including PM_{10}, $PM_{2.5}$, 8h-maximal O_3, NO_2, SO_2 and carbon monoxide (CO) were generated from the ChinaHighAirPollutants (CHAP) dataset (ground-based measurements, satellite remote sensing products, atmospheric reanalysis and model simulations) with using artificial intelligence which allows for the spatial and temporal variability of air pollution over the study period.[36-39] The ground-level concentrations of air pollutants were derived from the Atmospheric Composition Analysis Group, which estimates the ground-level concentrations through aerosol optical depth (AOD) by the NASA MODIS, MISR, and SeaWiFS satellite instruments. Air pollutants estimates were highly consistent with out-of-sample air pollutants measurements, with the cross-validation coefficient of determination (CV-R^2) of 0.9 for PM_{10}, 0.92 for $PM_{2.5}$, 0.87 for O_3, 0.84 for NO_2, 0.8 for CO, and 0.84 for SO_2, respectively. In the basic of the established model, we used monthly estimates in 2000−20 with a high- resolution of 1×1 km for PM_{10} and $PM_{2.5}$, and monthly estimates in 2013−20 with a spatial resolution of 10×10 km for the rest pollutants for data analysis.

Monthly individual-level exposures of PM_{10}, $PM_{2.5}$, 8h-maximal O_3, SO_2, NO_2 and CO were assigned to each subject based on the latitudes and longitudes of geocoded residential addresses which were picked up from the Baidu Map (https://api.map.baidu.com). By linking longitude and latitude coordinates of residential location to the nearest pollutants' grid, monthly mean concentrations of the 1 km grid-cell for $PM_{2.5}$ and PM_{10}, and 10-km grid-cell for the other four pollutants were assigned to each subject. We then calculated the monthly mean concentrations of air pollutants during 3 periods (Figure 2). In view of the three-month period normally be regarded to be the length of the gamete refresh cycle, we defined Period 1 as 90 days before oocyte retrieval. Considering potentially long-term adverse effects of pollutants on pregnancy outcomes, we defined Period 2 as one year and Period 3 as two years before oocyte retrieval. Because of unavailable data of SO_2, CO, NO_2 and O_3 before 2013 in this dataset, in Period 3, we merely calculated the exposure levels of $PM_{2.5}$ and PM_{10}.

2.5 Statistical Analysis

For descriptive analysis, personal characteristics are expressed as means (± standard deviation (SD)) and numbers (percentages, %) for quantitative variables Exposure data in different exposure periods are presented by the mean, SD, minimum, 25th percentile, median, 75th percentile and maximum.

We used multivariate logistic regression models to explore the associations of air pollutants with four binary pregnancy outcomes. We reported effect estimates (β), adjusted odds ratios (aORs) and 95% confidence intervals (CIs) associated with an interquartile range (IQR) increment of pollutants concentrations in the three Periods. We empirically considered confounders according to prior publication.[40] Before starting the main model, we used variance inflation factors (VIF) diagnostics to assess the multicollinearity of these covariates. We just found the VIF value between the type of transferred embryos and COS protocol exceeded 10 (VIF>10), and then used ridge regression to exclude the covariate of COS protocol in the basis of smaller absolute values of coefficients in this model. Finally, the following confounders were adjusted in the main models: age, BMI, season, educational level, employment status, duration of infertility, type of infertility, type of transferred embryos, number of transferred embryos and quality of transferred embryos.

We, meanwhile, performed stratified analyses in different subgroups. Before stratified analyses, in order to estimate the season-specific associations with the IVF pregnancy outcomes, we fitted separate models by season group [cold (October-March) and warm (April-September)]. Moreover, to identify the vulnerable population, we conducted stratified analyses by fitting separate models by type of transferred embryos (fresh/frozen), age (<32/⩾32 years), BMI [normal (18.5~23.9 kg/m^2) and abnormal (<18.5 or ⩾24 kg/m^2)], employment status (employed/unemployed), type of infertility (primary/secondary), education level (⩽junior high school, high school and ⩾ Junior college), and number of transferred embryos (one, two and three) to evaluate the potential effect modifications. We further compared the effect estimates of each air pollutant on pregnancy outcomes between the two group by calculating 95% CIs of the differences by using the formula of $(\hat{E}_1 - \hat{E}_2) \pm 1.96 * \sqrt{S\hat{E}_1^{\ 2} + S\hat{E}_2^{\ 2}}$, where $\hat{E}_1$ and $\hat{E}_2$ are the regression coefficients for subgroups, $S\hat{E}_1^{\ 2}$ and $S\hat{E}_2^{\ 2}$ are corresponding standard errors. We also used 2-sample *z*-tests with the formula of $(\hat{E}_1 - \hat{E}_2) / \sqrt{S\hat{E}_1^{\ 2} + S\hat{E}_2^{\ 2}}$ to test the statistical significance of these differences. For the sensitivity analyses, we fitted two-pollutant models by additionally controlling for air pollutants one by one to test the robustness of the estimated associations. Lastly, in order to assess the effects of the coronavirus disease 2019 (COVID-19) lockdowns, we conducted additional sensitivity analysis excluding the year 2020.

All statistical analyses were conducted in R software with version 4.2.2 (R Foundation for Statistical Computing, Vienna, Austria), and a *p*-value of <0.05 based on two-sided calculation was deemed as statistical significance.

3. Results

3.1 Descriptive Results

Of the 15,414 patients who underwent assisted reproductive technology in the center during 2013−20, a total of 11,968 eligible patients were included in formal analysis (Figure 1). Details on personal characteristics were summarized in Table 1. The mean age and BMI were 32.35 (± 5.81) years and 23.15 (± 3.53) kg/m^2, respectively, and 57.98% received frozen embryo transfers. Of 11,968 eligible patients, 45.62% and 39.05% resulted in a biochemical pregnancy and clinical pregnancy, respectively. Of 4,673 patients with clinical pregnancy, 77.49% resulted in live birth while 22.11% resulted in pregnancy loss. During the whole study period, no patient left the studied residential addresses. There were no statistically significant differences between included and excluded patients (data not shown).

Table 1. Characteristics of 11,968 patients undergoing assisted reproductive technology

Variables	Total participants	Biochemical pregnancy	Clinical pregnancy	Pregnancy loss [b]	Live birth [b]
N (%)	11968	5460 (45.62)	4673 (39.05)	1033 (22.11)	3621 (77.49)
Age (Mean (SD))	32.35 (5.81)	31.16 (5.10)	31.07 (5.03)	32.68 (5.72)	30.61 (4.72)
< 32 (%)	6069 (50.71)	3173 (58.11)	2744 (58.72)	461 (44.63)	2271 (62.72)
⩾ 32 (%)	5899 (49.29)	2287 (41.89)	1929 (41.28)	572 (55.37)	1350 (37.28)
BMI (Mean (SD))	23.15 (3.53)	23.17 (3.60)	23.17 (3.59)	23.52 (3.73)	23.07 (3.55)
Normal (%)	6983 (58.35)	3171 (58.08)	2719 (58.19)	570 (55.18)	2138 (59.04)
Abnormal (%)	4985 (41.65)	2289 (41.92)	1954 (41.81)	463 (44.82)	1483 (40.96)
Employment status (%)					
Employed	7457 (62.31)	3295 (60.35)	2806 (60.05)	628 (60.79)	2171 (59.96)
Unemployed	4511 (37.69)	2165 (39.65)	1867 (39.95)	405 (39.21)	1450 (40.04)
Education level (%)					
⩽ Junior high school	6301 (52.65)	2870 (52.56)	2429 (51.98)	564 (54.60)	1852 (51.15)
High school	2311 (19.31)	1041 (19.07)	911 (19.49)	187 (18.10)	722 (19.94)
⩾ Junior college	3356 (28.04)	1549 (28.37)	1333 (28.53)	282 (27.30)	1047 (28.91)
Type of infertility (%)					
Primary infertility	4964 (41.48)	2406 (44.07)	2063 (44.15)	388 (37.56)	1665 (45.98)
Secondary infertility	7004 (58.52)	3054 (55.93)	2610 (55.85)	645 (62.44)	1956 (54.02)
Duration of infertility (Mean (SD))	4.61 (3.46)	4.44 (3.21)	4.43 (3.19)	4.77 (3.76)	4.33 (3.00)
Embryo transfer type (%)					
Fresh	5029 (42.02)	2269 (41.56)	1949 (41.71)	432 (41.82)	1515 (41.84)
Frozen	6939 (57.98)	3191 (58.44)	2724 (58.29)	601 (58.18)	2106 (58.16)

Table 1 (*continued*)

Variables	Total participants	Biochemical pregnancy	Clinical pregnancy	Pregnancy loss [b]	Live birth [b]
Number of transferred embryos (%)					
One	3207 (26.80)	1693 (31.01)	1529 (32.72)	270 (26.14)	1248 (34.47)
Two	6803 (56.84)	3072 (56.26)	2577 (55.15)	605 (58.57)	1965 (54.27)
Three	1958 (16.36)	695 (12.73)	567 (12.13)	158 (15.30)	408 (11.27)
Stimulation protocol (%)					
Long GnRH-a protocol	2301 (19.23)	1116 (20.44)	937 (20.05)	179 (17.33)	758 (20.93)
Short GnRH-a protocol	195 (1.63)	53 (0.97)	45 (0.96)	19 (1.84)	26 (0.72)
GnRH antagonist protocol	2347 (19.61)	1022 (18.72)	897 (19.20)	217 (21.01)	678 (18.72)
Hormone replacement treatment protocol	6141 (51.31)	2795 (51.19)	2385 (51.04)	531 (51.40)	1840 (50.81)
Others [a]	984 (8.22)	474 (8.68)	409 (8.75)	87 (8.42)	319 (8.81)
Quality of transferred embryos (%)					
Good quality embryo	7373 (61.61)	3494 (63.99)	3023 (64.69)	630 (60.99)	2378 (65.67)
General embryo	4595 (38.39)	1966 (36.01)	1650 (35.31)	403 (39.01)	1243 (34.33)
Season (%)					
Cold	5258 (43.93)	2378 (43.55)	2043 (43.72)	436 (42.21)	1597 (44.10)
Warm	6710 (56.07)	3082 (56.45)	2630 (56.28)	597 (57.79)	2024 (55.90)
Quarter (%)					
Q1th	3163 (26.43)	1444 (26.45)	1225 (26.21)	262 (25.36)	958 (26.46)
Q2nd	3280 (27.41)	1519 (27.82)	1299 (27.80)	312 (30.20)	983 (27.15)
Q3rd	3278 (27.39)	1478 (27.07)	1277 (27.33)	281 (27.20)	993 (27.42)
Q4th	2247 (18.78)	1019 (18.66)	872 (18.66)	178 (17.23)	687 (18.97)

Abbreviations: SD, standard deviation; ART, assisted reproductive technology; BMI, body mass index; Q1th, the first quarter; Q2nd, the second quarter; Q3rd, the third quarter; Q4th, the fourth quarter; Long GnRH-a protocol, the long gonadotrophin-releasing hormone (GnRH)- agonist (-

a) protocol; Short GnRH- protocol, the short gonadotrophin-releasing hormone (GnRH)- agonist (-a) protocol; GnRH Antagonist, gonadotrophin-releasing hormone antagonist.

[a] Other protocols include short GnRH-agonist protocol, mild stimulation protocol and progestin-primed ovarian stimulation (PPOS) protocol.

[b] 4654 participants were included in analysis for pregnancy loss and live birth outcomes due to 19 losing of follow-up from 4673 clinical pregnancy cases.

On average, monthly mean concentrations of six criteria air pollutants in the three exposure periods (Period 1, Period 2 and Period 3) were presented in Table 2. Take Period 2 for instance, the monthly mean concentrations of PM_{10}, $PM_{2.5}$, O_3, CO, SO_2, and NO_2 were 91.04 μg/m^3, 56.36 μg/m^3, 91.45 μg/m^3, 0.89 mg/m^3, 17.35 μg/m^3, and 33.13 μg/m^3, respectively. Notably, the mean PM_{10} and $PM_{2.5}$ was all far beyond the 2021 WHO Air Quality Guidelines (annual mean for $PM_{2.5}$: 5 μg/m^3, and PM_{10}: 15 μg/m^3) (Figure S1). We also summarized the pollutants concentrations in Periods 1, 2 and 3 among participants of different health outcomes (Table S1).

We further analyzed the correlation of pollutants in Period 2 (Table S2). $PM_{2.5}$ concentration in Period 2 was positively correlated with that of PM_{10}, SO_2, CO and NO_2 (Spearman r = 0.981, 0.728, 0.715 and 0.220, respectively) while negatively correlated with that of O_3 (r = -0.495). O_3 was negatively correlated with all pollutants except NO_2. Other correlations between pollutants were shown in Table S2.

Table 2. Descriptive statistics of air pollutants concentrations in different exposure windows

Pollutants	Period [a]	Mean (SD)	Median (Min, Max)	IQR (P_{25}, P_{75})
PM_{10} (μg/m^3)	1	85.82 (28.85)	83.40 (21.23, 206.10)	40.02 (64.02, 104.03)
	2	91.04 (20.41)	89.38 (31.26, 165.84)	23.18 (78.31, 101.49)
	3	94.44 (19.54)	93.26 (32.25, 172.20)	26.33 (81.51, 107.84)
$PM_{2.5}$ (μg/m^3)	1	52.26 (21.47)	48.70 (9.47, 144.47)	30.92 (35.52, 66.43)
	2	56.36 (13.10)	55.43 (16.19, 108.68)	15.18 (48.10, 63.28)
	3	58.59 (12.35)	57.81 (16.99, 108.32)	17.23 (50.34, 67.57)
O_3-8 h (μg/m^3)	1	96.68 (28.67)	96.65 (20.41, 191.15)	45.14 (73.77, 118.91)
	2	91.45 (18.43)	95.09 (28.35, 125.33)	27.05 (79.68, 106.73)
CO (mg/m^3)	1	0.86 (0.21)	0.82 (0.27, 2.45)	0.26 (0.71, 0.97)
	2	0.89 (0.14)	0.87 (0.35, 1.93)	0.20 (0.78, 0.98)
SO_2 (μg/m^3)	1	16.45 (8.72)	14.57 (4.02, 77.58)	10.65 (9.87, 20.52)
	2	17.35 (7.97)	15.86 (4.93, 62.93)	10.91 (11.08, 21.99)
NO_2 (μg/m^3)	1	31.98 (10.36)	30.48 (6.68, 76.21)	13.59 (24.56, 38.15)
	2	33.13 (6.88)	32.79 (9.08, 62.57)	5.92 (29.69, 35.61)

Abbreviations: $PM_{2.5}$, particulate matter with an aerodynamic diameter of 2.5 μm or less; PM_{10}, particulate matter with an aerodynamic diameter of 10 μm or less; SO_2, sulfur dioxide; CO, carbon monoxide; O_3-8 h, 8 h maximal ozone; NO_2, Nitrogen dioxide; SD, standard deviation; Min, minimum; Max, maximum; IQR, interquartile range; P_{25}, the 25th percentile; P_{75}, the 75th percentile.

[a] Periods 1–3 indicate the exposure windows shown in Figure. 2. Period 1, 90 days prior to oocyte retrieval; Period 2, one year prior to oocyte retrieval; Period 3, two years prior to oocyte retrieval.

3.2 Regression Results

As shown in Table 3, we listed multivariate-adjusted ORs of four ART pregnancy outcomes perturbed by ambient air pollutants exposures during three exposure windows. Overall, we found negative and significant relationships of exposures to PM_{10}, $PM_{2.5}$, CO and SO_2 with health outcomes in different exposure windows, whereas O_3 or NO_2 exposure presented significantly inverse associations except for live birth. To be specific, we observed associations of exposures to PM_{10} and $PM_{2.5}$ in Periods 2 and 3 with a low likelihood of achieving pregnancy in undergoing embryo transfers while null associations with pregnancy loss and live birth. For $PM_{2.5}$, an IQR increase in $PM_{2.5}$ in Period 2 (15.18 $\mu g/m^3$) was related to 7.82% and 7.51 % decrements in the likelihood of clinical pregnancy (aOR: 0.92, 95% CI: 0.88–0.97) and biochemical pregnancy (aOR: 0.92, 95% CI: 0.88–0.97), respectively, while during Period 3 (17.23 $\mu g/m^3$), it was related to a respective 10.00% and 9.63 % reduction in the likelihood of clinical pregnancy (aOR: 0.90, 95% CI: 0.85–0.95) and biochemical pregnancy (aOR: 0.90, 95% CI: 0.85–0.96). The similar results were also observed for CO and SO_2 (Table 3). For instance, for CO, the odds were decreased by 10.6% (aOR: 0.89, 95% CI: 0.84–0.95) for clinical pregnancy and by 8.66% (aOR: 0.91, 95% CI: 0.86–0.97) for biochemical pregnancy. While null associations were observed for pregnancy loss and live birth in Period 2. For O_3 exposure, in Period 1, there were significant and negative relationships of O_3 with live birth, while significant and positive associations with the rest health outcomes and we observed similar results for NO_2 in Period 2.

Table 3. Relationship between exposure to ambient air pollutants and pregnancy outcomes during 3 exposure windows among patients undergoing assisted reproductive technology [a]

Period [b]	Pollutant	Biochemical Pregnancy		Clinical pregnancy		Pregnancy Loss		Live Birth	
		β	aOR (95% *CI*)	β	aOR (95% *CI*)	β	aOR (95% *CI*)	β	aOR (95% *CI*)
Period 1	PM_{10}	-0.0010	0.96 (0.91, 1.01)	-0.0012	0.95 (0.90, 1.01)	-0.0008	0.97 (0.87, 1.07)	0.0008	1.03 (0.93, 1.14)
	$PM_{2.5}$	-0.0013	0.96 (0.91, 1.02)	-0.0017	0.95 (0.90, 1.01)	-0.0016	0.95 (0.86, 1.06)	0.0016	1.05 (0.94, 1.17)
	O_3-8 h	0.0017	1.08 (1.01, 1.15)	0.0023	1.11 (1.04, 1.19)	0.0030	1.14 (1.01, 1.30)	-0.0030	0.87 (0.77, 0.99)
	CO	-0.1658	0.96 (0.91, 1.01)	-0.2420	0.94 (0.89, 0.99)	-0.0577	0.99 (0.90, 1.08)	0.0577	1.01 (0.93, 1.11)
	SO_2	-0.0110	0.89 (0.85, 0.94)	-0.0143	0.86 (0.81, 0.91)	-0.0002	1.00 (0.91, 1.10)	0.0002	1.00 (0.91, 1.10)
	NO_2	0.0038	1.05 (1.00, 1.11)	0.0037	1.05 (1.00, 1.11)	-0.0016	0.98 (0.89, 1.08)	0.0016	1.02 (0.93, 1.13)
Period 2	PM_{10}	-0.0025	0.94 (0.90, 0.99)	-0.0026	0.94 (0.90, 0.99)	0.0003	1.01 (0.92, 1.10)	-0.0003	0.99 (0.91, 1.08)
	$PM_{2.5}$	-0.0051	0.92 (0.88, 0.97)	-0.0054	0.92 (0.88, 0.97)	0.0016	1.02 (0.94, 1.12)	-0.0016	0.98 (0.89, 1.07)
	O_3-8 h	0.0058	1.17 (1.09, 1.25)	0.0076	1.23 (1.15, 1.32)	0.0038	1.10 (0.97, 1.25)	-0.0038	0.91 (0.80, 1.03)
	CO	-0.4541	0.91 (0.86, 0.97)	-0.5619	0.89 (0.84, 0.95)	-0.1222	0.98 (0.87, 1.09)	0.1222	1.02 (0.92, 1.14)
	SO_2	-0.0100	0.90 (0.84, 0.95)	-0.0121	0.88 (0.82, 0.93)	-0.0026	0.97 (0.87, 1.09)	0.0026	1.03 (0.92, 1.15)
	NO_2	0.0061	1.04 (1.00, 1.07)	0.0062	1.04 (1.00, 1.07)	0.0072	1.04 (1.00, 1.09)	-0.0072	0.96 (0.91, 1.00)
Period 3	PM_{10}	-0.0031	0.92 (0.87, 0.97)	-0.0031	0.92 (0.87, 0.97)	-0.0009	0.98 (0.89, 1.08)	0.0009	1.02 (0.93, 1.13)
	$PM_{2.5}$	-0.0059	0.90 (0.85, 0.96)	-0.0061	0.90 (0.85, 0.95)	-0.0006	0.99 (0.89, 1.10)	0.0006	1.01 (0.91, 1.12)

Abbreviations: $PM_{2.5}$, particulate matter with an aerodynamic diameter of 2.5 μm or less; PM_{10}, particulate matter with an aerodynamic diameter of 10 μm or less; SO_2, sulfur dioxide; CO, carbon monoxide; O_3-8 h, 8 h maximal ozone; NO_2, Nitrogen dioxide.

[a] Effects on pregnancy outcomes are presented as adjusted OR (aOR) and 95% confidence intervals (CIs) per IQR increase in concentrations of each air pollutant.

[b] Periods 1, 2 and 3 indicate the exposure periods shown in Figure. 2. Period 1, 90 days prior to oocyte retrieval; Period 2, one year prior to oocyte retrieval; Period 3, two years prior to oocyte retrieval.

Because we found these associations occurred in Period 2 were more evident, we derived the risk estimates during this exposure window in subsequent analyses. In stratification analyses, the odds of PM_{10} and $PM_{2.5}$ in Period 2 with biochemical pregnancy and clinical pregnancy were lower during warm season than cold season [(for biochemical pregnancy: PM_{10}: 0.93 vs. 0.96 per IQR; $PM_{2.5}$: 0.91 vs. 0.94 per IQR; CO: 0.86 vs. 0.98 per IQR; and SO_2: 0.88 vs. 0.91 per IQR); for clinical pregnancy: PM_{10}: 0.93 vs. 0.95 per IQR; $PM_{2.5}$: 0.91 vs. 0.94 per IQR; CO: 0.84 vs. 0.96 per IQR; and SO_2: 0.85 vs. 0.90 per IQR)] (Figure 3). The between-group differences for $PM_{2.5}$ in Period 1 and 2 and PM_{10} in Period 2 on live birth and pregnancy loss reached statistical significance. We observed the same results during Periods 3 (Table S4). We further observed higher decreased odds in warm season for CO in Period 2 ($P<0.05$ for all comparisons on biochemical pregnancy and clinical pregnancy) and SO_2 in Periods 1 and 2 ($P<0.05$ for all comparisons on pregnancy loss and live birth) (Figure 3 and Table S3). Further stratification by age of patients revealed significantly stronger associations for lower rates of clinical pregnancy in patients aged 32 years or younger with exposures to PM_{10}, $PM_{2.5}$ and SO_2 in Period 2 (Figure 4). We also observed similar results in the other two exposure windows (Table S5 and Table S6). Although all between-strata differences for O_3 exposure in Period 1 were not statistically significant, we still observed that patients aged 32 years or older tended to have lower odds for live birth (aOR: 0.81) and higher odds for pregnancy loss (aOR: 1.23) (Table S5). Another stratification by type of embryo (fresh vs. frozen) revealed that negative associations of PM_{10}, $PM_{2.5}$, SO_2 and CO with biochemical/clinical pregnancy were only observed in patients with frozen embryo transfer (Figure 5). Take SO_2 in Period 2 for instance, the risks for biochemical pregnancy and clinical pregnancy were significantly lower among patients receiving frozen embryo transfer than those with fresh embryo transfer (biochemical pregnancy: 0.85 vs. 0.98 per IQR; clinical pregnancy: 0.83 vs. 0.98 per IQR, $P<0.05$ for all comparisons) (Figure 5). Similar associations of PM_{10} and $PM_{2.5}$ were also observed in Period 2 and 3 (Figure 5 and Table S8). In contrast to the aforementioned findings, we found O_3 and NO_2 exposures in Period 1 and 2 were associated with a higher possibility of biochemical pregnancy and clinical pregnancy in frozen embryo transfer patients, while with a lower possibility of O_3 on live birth in fresh embryo transfer patients (Figure 5 and Table S7). Further stratification by the type of infertility revealed that higher PM_{10}, $PM_{2.5}$, SO_2 and CO exposures were associated with lower odds of biochemical pregnancy and clinical pregnancy in primary infertility subjects than those who were secondary infertility; inversely, higher O_3 exposure was correlated with a higher possibility of biochemical pregnancy and clinical pregnancy (Table S9). Patients who had normal BMI, were in employment, or had a high school education showed larger risks of adverse pregnancy outcomes; there was a significant between-strata difference for PM_{10} on biochemical pregnancy in patients with high school level (P = 0.049) (Table S10 and Table S12).

Stratification analysis by number of embryo transfer showed lower odds of biochemical pregnancy and clinical pregnancy in patients who transferred one embryo in Period 2 (Table S13). We also observed significant between-strata differences in clinical pregnancy for SO_2 during Period 2 among patients with transferred one embryo (*P* = 0.015) (Table S13).

We further conducted two-pollutant regression models by controlling for two pollutants with a correlation coefficient less than 0.7 (Table S2). The results from two-pollutant models for the effects of NO_2, CO and SO_2 did not substantially change, suggesting minimal confounding from each other (Table S14). However, after controlling for O_3, the magnitude (i.e., the size of ORs) of the associations of PM_{10}, $PM_{2.5}$, CO and SO_2 concentrations increased (Table S14).

Lastly, to explore the effects of COVID-19 lockdowns, we conducted another sensitivity analysis excluding the year 2020 (Table S15). After excluding the year 2020, the results did not significantly change, indicating the robustness of our main regression models.

Discussion

This retrospective longitudinal cohort study demonstrated that significant relationships between ambient air pollutants (PM_{10}, $PM_{2.5}$, SO_2 and CO) exposures during the period of interest and increased risks of adverse pregnancy outcomes in patients undergoing ART, especially during one entire year exposure before oocyte retrieval. Surprisingly, throughout the analyses, O_3 and NO_2 had a significantly positive relationship with biochemical pregnancy and clinical pregnancy, suggesting it plays seemingly potential ‘protective’ role in the risk of pregnancy outcomes. We found stronger associations among patients aged <32 years, with frozen transferred embryos, normal BMI, employed status, a high school degree, primary infertility, transferred one embryo and in the warm season. To our knowledge, this is one of the limited retrospective cohort investigations that has systematically explored the roles of outdoor air pollutants exposure in the risk of ART results by using individual-level database, and this is the first study in Eastern China that has explored the modification effects of seasons in pollutants-associated ART pregnancy outcomes. And our findings also provided additional evidence to the unfavorable reproductive effects of air pollution and the relevant time course which underscores the continuing efforts for controlling the air pollution.

In this study, we observed that significant associations of $PM_{2.5}$, PM_{10}, SO_2 and CO exposures with decreased likelihood of pregnancy, which is consistent with existing publication.[21,41-43] For example, an exploratory retrospective cohort study of 1,139 IVF attempts with 518 clinical pregnancy in Chengdu from 2014 to 2019 showed that $PM_{2.5}$, PM_{10}, NO_2, SO_2 and CO was negatively associated with biochemical pregnancy and clinical pregnancy rate, while O_3 was positively associated with the two outcomes.[42] Nevertheless, two research reported null association of $PM_{2.5}$ with pregnancy loss, live birth and clinical pregnancy from oocyte retrieval to embryo transfer.[31,44] As for O_3 and NO_2, we observed contrast results as a positive effect of O_3 and NO_2 but a negative effect of $PM_{2.5}$. In addition, compared with the other two exposure windows, the impacts of particulate matter (PM) ($PM_{2.5}$ and PM_{10}) were more evident in Period 3, suggesting that long-term exposure to $PM_{2.5}$ and PM_{10} is likely to cause biological changes such as systemic oxidative stress, endocrine dysfunction and epigenetic changes,[45] which may further lead to adverse maternal effects such as decreased placental function.[46]

Interestingly, the correlation between O_3 concentration and biochemical/clinical pregnancy is opposite to the results for other pollutants. Specifically, exposure to O_3 increased the likelihood of biochemical pregnancy and clinical pregnancy in both single- and two-pollutant models, which is in line with two previous investigations performed in China.[21,42] In fact, the formation of near-surface O_3 is another secondary pollutant in air formed from the interaction of precursor pollutants (i.e., nitrogen oxide, volatile organic compounds, and CO) with sunlight.[47] Thus, negatively correlation of near-surface O_3 concentration with concentrations of those precursors. As for NO_2, two studies reported adverse correlation between NO_2 exposure and intrauterine pregnancy in ART treatment,[43,48] while another publication observed null association.[31] Interestingly, we observed positive association of NO_2 with ART outcomes. Thus, the contradictory results of NO_2 and O_3 for ART pregnancy outcomes need further investigation to confirm.

The stratified analyses revealed significant evidence of type of embryo transfer heterogeneity in the relationships of PM_{10}, $PM_{2.5}$, SO_2, and CO exposures with health outcomes in patients receiving frozen embryo transfers (FET). In line with this finding, one recent publication indicated that participants who received FET were more affected following ambient air pollution.[21] One retrospective cohort study conducted in Xiamen, China also found that SO_2 and O_3 exposures were significantly correlated to live birth rates in frozen cycles.[26] However, Shi et al found that the higher level of PM_{10} (the median of PM_{10} was 50 μg/m^3 approximately) could decrease the rates of live birth in fresh cycles but not affect the FET outcomes.[20] We speculate that the different exposure concentration and the inconsistent method for endometrium preparation in ovulatory patients may lead to this discrepancy.[49]

Female reproductive aging is an important factor leading to adverse pregnancy outcomes, as the lower oocyte quantity and quality as well as uterine and placental dysfunctions with age will further result in infertility.[50] We also observed significant difference between age groups. In our study, stronger associations of pregnancy outcomes were observed in patients with aged < 32 years but not in above age 32. Consistent with this finding, a retrospective study included 2,020 IVF-FET patients in Zhengzhou of China reported that the detrimental impacts of pollutants were much apparent in participants below age 32 years.[51] The abovementioned findings indicated that younger participants especially those under the age of 32 might be more subjected to air pollution.

Therefore, these effects on IVF/ICSI pregnancy outcomes following air pollutants exposures are possibly masked by age-related adverse effects,[52] which means vulnerable groups especially these younger participants should be paid more attention to air pollution and the corresponding preventative measures should be taken to minimize the harm induced by environmental pollution.

The modification of season on the association of air pollution with IVF/ICSI pregnancy outcomes has not yet been fully elucidated. Interestingly, we observed significant evidence of season heterogeneity in the associations between pollutants exposure and pregnancy outcomes in ART patients. Specifically, PM_{10}, $PM_{2.5}$ and CO exposures were significantly associated with decreased odds of biochemical pregnancy and clinical pregnancy in warm seasons but not in cold seasons. Some studies have reported that heat exposure related to climate change could cause the higher risk of numerous adverse pregnancy outcomes (preterm birth, ect) in natural conception patients.[53-55] Unlike ours and the abovementioned findings, Guo and colleagues observed stronger associations between $PM_{2.5}$ and pregnancy outcomes in cold season;[56] and Correia and colleagues found that warmer temperature at oocyte retrieval could increase the odds of clinical pregnancy.[57] On one hand, this discrepancy is possibly resulted from seasonal and geographical variations of pollutants exposure levels, different meteorological conditions and different living habits of subjects. On the other hand, seasonality factors are correlated with ovarian function and oocyte quality. Further, stronger associations in primary infertility patients or in employed patients were also found in our study. It is probable that employed participants usually spend more time outside during the day and undergo emotional stress like anxiety, further making them be more liable to be affected by ambient air pollution than those unemployed participants. Moreover, area level SES and urbanicity can also affect urban pollution levels.

Moreover, we found significant associations in patients who underwent single embryo transfer but almost null associations in patients following two or three embryo transfers, which is partly explained by the higher rates of pregnancy and live birth after two/three transferred embryos, and in turn making the contributions from air pollutants on ART pregnancy outcomes less relevant. Together with the similar finding from one study of Shanghai, China, this phenomenon implied that patients following single transferred embryo tend to be larger risks of adverse pregnancy outcomes following air pollutants exposures. Another interestingly finding is that we found normal-BMI patients with lower rates of biochemical/clinical pregnancy perturbed by air pollutants exposure, while null association was observed in abnormal-BMI (overweight or underweight) patients. As far as we know, few literatures have addressed this critical question. Some reported that the BMI of ART women did not affect clinical pregnancy outcomes and live birth rates,[58-60] while others reported underweight women had lower rates of clinical pregnancy[61] and live birth as well as a higher rate of pregnancy loss;[62] and thus suggests the necessity of further research to confirm the role of BMI. To date, the role of women's educational level in the air pollution associated with assisted reproductive outcomes is still scare and further investigations were warranted. What’s more we found the examined pollutants except O_3 exposures were negatively correlated to biochemical pregnancy and clinical pregnancy in group with a high school education. However, Cantarutti et al reported high education women who had lower adverse pregnancy outcomes were at a higher likelihood of some disadvantageous neonatal outcomes.[63] In sum, despite our findings from these stratified analyses were interesting, they must be interpreted with caution and need independent confirmations.

Due to climbing rates of infertility, our results may help explain the women’s reproductive health that were responsible for the adverse effects of air pollution, which further help physicians and vulnerable individuals to better manage the reproductive health and to take proper precautions. Possible mitigation actions include avoiding going outdoors when encountering days of heavy air pollution or in the warm season especially in heavy polluted days, staying indoors with opening air purification system, changing unhealthy lifestyle, strengthening reproductive viability monitoring, timely regulating treatment program, using preventive adverse pregnancy outcomes medicine, and extending preventive measures against air pollution at least one year before receiving ART treatment. In addition, more need should be paid to those younger women child-bearing age, employed women and women with transferred one embryo and FET.

This study has several strengths. First, for all we know, this was one of the limited investigations to discuss the chronic relationship of outdoor air pollution with ART results in mainland China. Second, the air pollution data at the monthly time scale which were generated from the big dataset by using artificial intelligence with a view of the spatial-temporal variability of air pollution. We then linked residential locations by the longitude and latitude coordinates, minimizing mismatch of exposures and outcomes and assisting in characterizing the lag pattern at submonthly timescales. Third, our health data of IVF or ICSI patients were obtained from standardized, validated, and detailed clinical records covering major patients of Hefei. And the large sample size with more than 10,000 contributes to increasing the statistical certainty in the estimation of the effect. Forth, we performed a wide variety of stratified analyses which allows us to relatively comprehensively detect the potential modifiers in the associations.

There were also important limitations that must be taken into account. First, only health data of one hospital in Hefei were included, which may have made our results less representativeness of the study population and limited the generalization to other Chinese population and to population from other areas. Second, our results should be interpreted with caution due to the retrospective nature of the study design. Future well-designed studies such as prospective cohort study should be performed to confirm our current findings. Third, exposure misclassification may be unavoidable due to lack of personal monitoring device and time-location activity pattern information (i.e., exposure during commuting and indoor/outdoor duration). It would be too costly and logistically prohibitive to conduct detail exposure measurement in a large population study as ours. However, our exposure predictions assigned at a 1x1 km resolution potentially minimized the magnitude of such misclassified exposures. Finally, unavailable information on confounders (i.e., smoking, alcohol intake and household heating) and other area-specific indicators (i.e., greenness and urbanicity) limited opportunity to identify more interesting findings.

Conclusions

This individual-level retrospective longitudinal cohort study found that ambient air pollutants exposures were significantly associated with increased adverse pregnancy outcomes among women undergoing ART. These observed associations were more evident in one year exposure before oocyte retrieval. The aforementioned results were more obvious in women who were below age 32 years, employed person, had normal BMI, had transferred one embryo, in FET, or in warm season. The evidence presented herein suggests the need for vigilance regarding to air pollution among younger women of child-bearing age and FET and women who are planning to have a baby at least one year before undergoing ART. Considering the high infertility rates in China, our results also provided significant evidence for modifiable environment stimulus of adverse ART adverse pregnancy outcomes that would help for prevention in high-risk individuals with women needing ART.

Abbreviations

ART	Assisted reproductive technology
$PM_{2.5}$	Fine particulate matter
PM_{10}	Suspended particulate matter
O_3	Ozone
SO_2	Sulfur dioxide
NO_2	Nitrogen dioxide
CO	Carbon oxide
CHAP	ChinaHighAirPollutants
BMI	Body mass index
WHO	World Health Organization
IVF	In vitro fertilization
ICSI	Intracytoplasmic sperm injection
FET	Frozen embryo transfer
COS	Controlled ovarian stimulation
HCG	Human chorionic gonadotropin
AOD	Aerosol optical depth
SD	Standard deviation
aORs	Adjusted odds ratios
CIs	Confidence intervals
β	Estimates
IQR	Interquartile range
VIF	Variance inflation factors

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CRediT authorship contribution statement

Miao Fang: Methodology, Formal analysis, Investigation, Software, Writing -original draft. **Qi Guo:** Formal analysis, Investigation, Writing -original draft. **Cunzhong Jiang:**

Data curation. **Lin Miao:** Methodology. **Liyan Yang:** Validation. **Zexi Wu**: Software. **Xiangyu Yao:** Visualization. **Feng Ni:** Conceptualization, Resources, Supervision, Project administration, Writing – review & editing. **Zhijing Lin:** Conceptualization, Methodology, Supervision, Funding acquisition, Writing – review & editing. **Dexiang Xu:** Resources, Supervision.

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Availability of data and materials

The data generated in this study are not publicly available due to privacy or ethical restrictions.

Declaration

Ethics approval and consent to participate

This study was approved by the Biomedical Institutional Review Board of the Anhui Medical University, with a waiver of informed consent.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Figure Legends

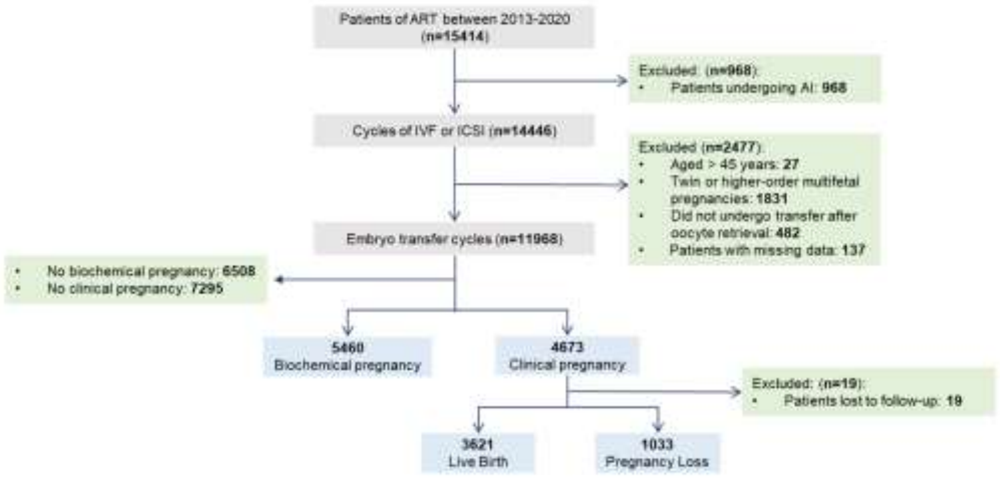

Figure 1. The flow diagram of the participants' enrollment. Abbreviations: ART, assisted reproductive technology; IVF, In vitro fertilization; ICSI, intracytoplasmic sperm injection; AI, artificial insemination.

Figure 2. The defined exposure windows of ART stages for this study.

Abbreviations: ART, assisted reproductive technology.

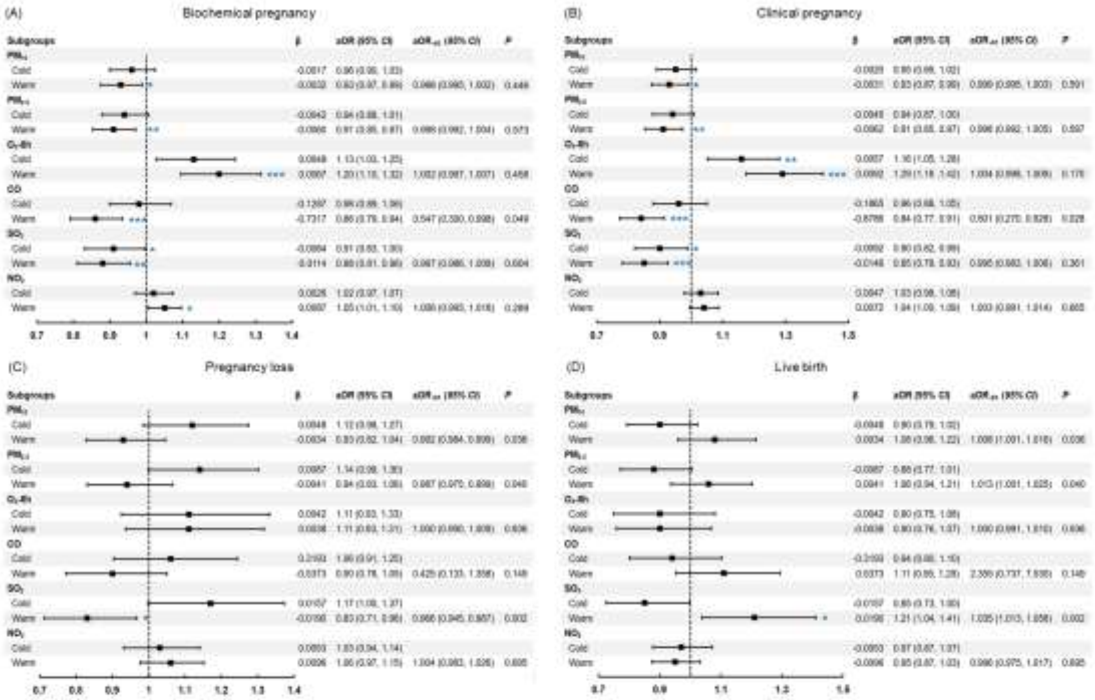

Figure 3. Relationship of air pollutants with pregnancy outcomes in patients undergoing assisted reproductive technology during Period 2 stratified by season. The associations are presented as adjusted odds ratio (OR) and 95% confidence interval (CI) of pregnancy outcomes associated with each interquartile range increase in air pollutants concentrations during Period 2 stratified by season [cold (October to March; n=5,258) vs warm (April to September; n=6,710)]. aOR dif. represents the differences between subgroups. *(*P*<0.05), **(*P*<0.01) and ***(*P*<0.001) indicate statistically significant association between exposures to six air pollutants and pregnancy outcomes in each group. *P* < 0.05 means significant between-subgroup difference. Abbreviations as in Table 2.

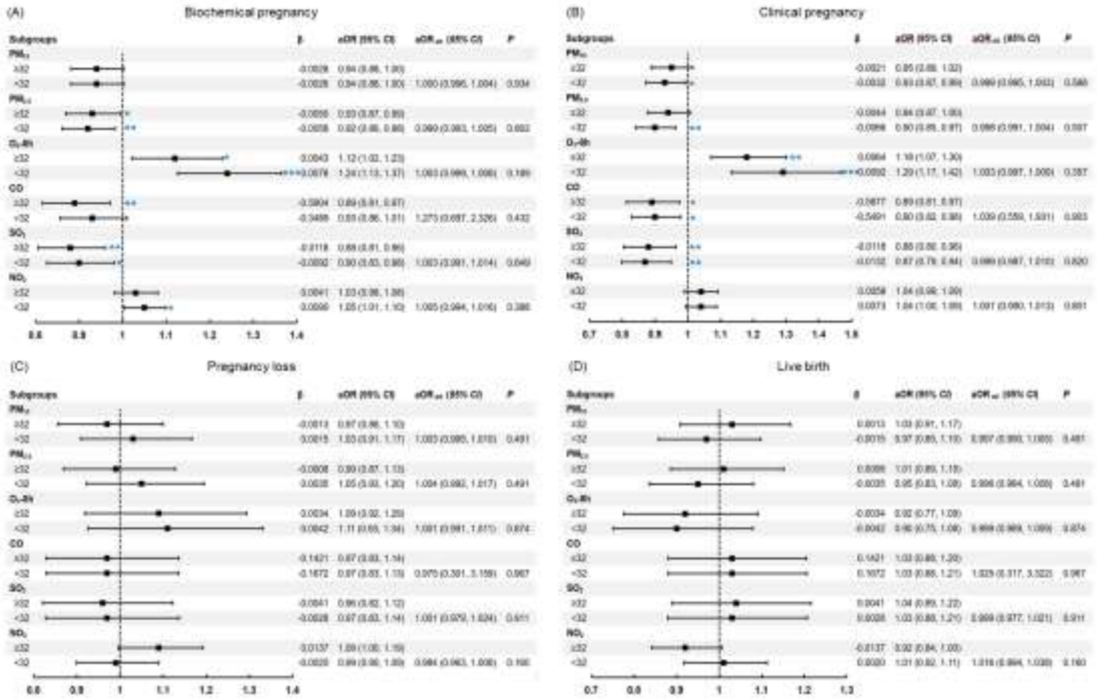

Figure 4. Relationship of air pollutants with pregnancy outcomes in patients undergoing assisted reproductive technology during Period 2 stratified by female age. The associations are presented as adjusted odds ratio (OR) and 95% confidence interval (CI) of pregnancy outcomes associated with each interquartile range increase in air pollutants concentrations during Period 1 stratified by age [<32 (n=6,069) vs ⩾32 (n=5,899)]. aOR dif. represents the differences between subgroups. *(*P*<0.05), **(*P*<0.01) and ***(*P*<0.001) indicate statistically significant association between exposures to five air pollutants and pregnancy outcomes in each group. *P* < 0.05 means significant between-subgroup difference. Abbreviations as in Table 2.

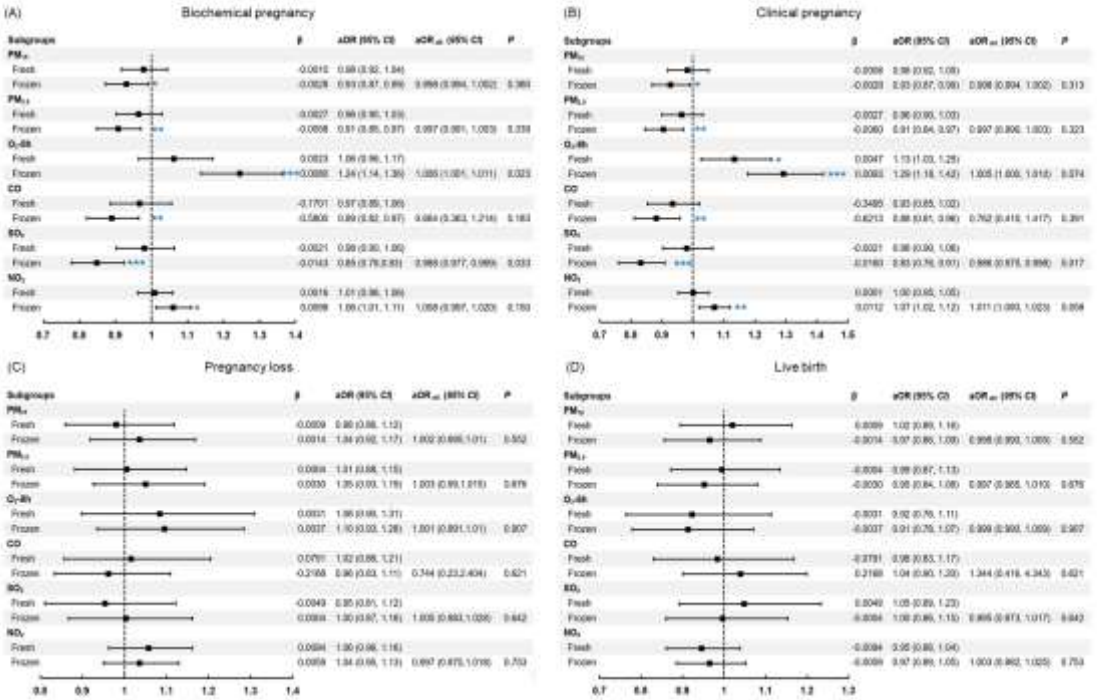

Figure 5. Relationship of air pollutants with pregnancy outcomes in patients undergoing assisted reproductive technology during Period 2 stratified by the type of embryo transfer. The associations are presented as adjusted odds ratio (OR) and 95% confidence interval (CI) of pregnancy outcomes associated with each interquartile range increase in air pollutants concentrations during Period 2 stratified by type of embryo transfer [fresh (n=5,029) vs frozen (n=6,939)]. aOR dif. represents the differences between subgroups. *(*P*<0.05), **(*P*<0.01) and ***(*P*<0.001) indicate statistically significant association between exposures to five air pollutants and pregnancy outcomes in each subgroup. *P* < 0.05 means significant between-subgroup difference. Abbreviations as in Table 2.

Supplementary Files

- SupplementaryMaterial.docx